

Appl. No. 09/552,461  
Amdt. dated January 24, 2004  
Reply to Office Action of Feb. 24, 2003

**Amendment to the Claims:**

Claim 12 (currently amended) A method for detecting a protein of known sequence comprising the steps of:

determining an optimal immunobiologically active linear epitope of said protein of known sequence, wherein said protein is comprised of a plurality of amino acids, and wherein said optimal immunobiologically active linear epitope is characterized by a hydrophobic-hydrophilic-hydrophobic (Ho-Hi-Ho) motif determined by a method comprising the steps of:

assigning an average hydropathy value to each of said plurality of amino acids of said protein of known sequence;  
generating a hydrophilicity plot using said average hydropathy value;

fitting each of a plurality of curve segments of said hydrophilicity plot to one of a plurality of a negative cosine functions, wherein one of a plurality of a specific period number values of said plurality of negative cosine functions equates to a particular number of amino acids in one of said plurality of curve segments, said period number value increasing within a predetermined chosen period number range after a sequential lagging of each of said plurality of curve segments through said hydrophilicity plot thereby providing a fit-correlation value for

Appl. No. 09/552,461  
Amdt. dated January 24, 2004  
Reply to Office Action of Feb. 24, 2003

each of said plurality of curve segments across said known sequence of said protein when using one of said plurality of said period number values;

generating a potential Ho-Hi-Ho epitope set for each of said plurality of specific period number values within said chosen period number range, wherein said potential Ho-Hi-Ho epitope set contains at least one potential Ho-Hi-Ho epitope in which said fit-correlation value is positive;

ranking each of said potential Ho-Hi-Ho epitopes of said potential Ho-Hi-Ho epitope set and assigning a ranking value to each of said potential Ho-Hi-Ho epitopes according to said fit-correlation value, wherein said potential Ho-Hi-Ho epitope with a highest positive fit-correlation value is ranked number one, thereby providing a ranked Ho-Hi-Ho potential epitope for each of said plurality of specific period number values;

examining said ranking value of each of said potential Ho-Hi-Ho epitopes relative to said hydrophilicity plot to determine at least one potential Ho-Hi-Ho epitope set that exhibits alternating positioning around an equilibrium position, wherein a plurality of said ranking values of said potential Ho-Hi-Ho epitopes converge towards or diverge away from said equilibrium position; and

Appl. No. 09/552,461

Amdt. dated January 24, 2004

Reply to Office Action of Feb. 24, 2003

designating each of said potential Ho-Hi-Ho epitopes, wherein said ranking values exhibit a most alternating ranking value that converges or diverges from said equilibrium position as said optimal immunobiologically active epitope wherein a numeric value of amino acid in said potential Ho-Hi-Ho epitopes is equal to one of said plurality of specific period number values of said negative cosine function;

synthesizing at least one peptide corresponding to at least one of said optimal immunobiologically active linear epitope;

creating at least one antisera against said synthesized peptides corresponding to at least one of said optimal immunobiologically active linear epitope;

providing a sample to be analyzed for said protein of known sequence;

contacting said sample with said at least one antisera; and

detecting a binding of said antisera to said protein of said sample, thereby indicating presence of said protein in said sample.

Claim 19. (currently amended) A method for detecting a protein of known sequence comprising the steps of:

determining an optimal immunobiologically active linear peptide epitope of said protein, wherein said protein is comprised of a plurality of amino acids, wherein said optimal immunobiologically active linear peptide

Appl. No. 09/552,461  
Amdt. dated January 24, 2004  
Reply to Office Action of Feb. 24, 2003

epitope is characterized by a Hydrophobic-Hydrophilic-Hydrophobic (Ho-Hi-Ho) motif determined by method comprising the steps of:

fitting a hydrophilicity/hydrophobicity plot generated for said protein of known sequence to a mathematically generated continuous curve thereby generating at least one potential Ho-Hi-Ho epitope set which includes at least one ranked potential Ho-Hi-Ho epitope, wherein one of a plurality of a numeric value is assigned to each of said potential Ho-Hi-Ho epitope set corresponding to a fit-correlation value of said hydrophilicity/hydrophobicity plot to said mathematically generated continuous curve, wherein said mathematically generated continuous curve has a period equal to a number of amino acids corresponding to length of said potential Ho-Hi-Ho epitope, said mathematically generated curve having a maximum positive value;

positioning said at least one ranked potential Ho-Hi-Ho epitope on said hydrophilicity/hydrophobicity plot to determine an oscillating behavior of said plurality of numeric values of said at least one ranked potential Ho-Hi-Ho epitope; and

deeming each of said plurality of ranked potential Ho-Hi-Ho epitope that exhibits a most alternating position about an equilibrium position when juxtaposed on said hydrophilicity/hydrophobicity plot as said optimal immunobiologically active linear peptide

Appl. No. 09/552,461

Amdt. dated January 24, 2004

Reply to Office Action of Feb. 24, 2003

epitope, wherein said optimal immunobiologically active linear peptide epitope and its optimal length corresponds to a number of amino acids in a set of ranked potential Ho-Hi-Ho epitopes; synthesizing at least one peptide corresponding to at least one of said optimal immunobiologically active linear epitope; creating at least one antisera against said synthesized peptides corresponding to at least one of said optimal immunobiologically active linear epitope; providing a sample to be analyzed for said protein of known sequence; contacting said sample with said at least one antisera; and detecting a binding of said antisera to said protein of said sample, thereby indicating presence of said protein in said sample.

Claim 21(New) The method of claim 12, wherein said protein is selected from a group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IFN- $\alpha$ , IFN- $\beta$ , IFN- $\gamma$ , CD2, CD3, CD4, CD5, CD8, CD11A, CD11b, CD11c, CD16, CD18, CD21, CD28, CD32, CD34, CD35, CD40, CD44, CD45, CD54, CD56, K2, K1, P $\beta$ , O $\alpha$ , M $\alpha$ , M $\beta$ 2, M $\beta$ 1, LMP1, TAP2, LMP7, TAP1, O $\beta$ , I $\alpha$  $\beta$ , I $\alpha$  $\alpha$ , I $\epsilon$  $\beta$ , I $\epsilon$  $\beta$ 2, I $\epsilon$  $\alpha$ , CYP21, C4B, CYP21P, C4A, BF, C2, HSP, G7a/b, TNF- $\alpha$ , TNF- $\beta$ , D, L Qa, T1a, COL11A2, DP $\beta$ 2, DP $\alpha$ 2, DP $\beta$ 1, DP 1, DN 2, DQ 3, DQ 1, DQ DR , DR , HSP-70, HLA-B, HLA-C, HLA-X, HLA-E, HLA-J, HLA-A, HLA-H, HLA-G, HLA-F, NGF, somatotropin, somatomedins, parathormone, FSH, LH, EGF, TSH, TSH-releasing factor, HGH, GRHR, PDGF, IGF-I, IGF-II, TGF- $\beta$ , GM-CSF, M-

Appl. No. 09/552,461  
 Amdt. dated January 24, 2004  
 Reply to Office Action of Feb. 24, 2003

CSF, G-CSF1, erythropoietin, -IIIG, 4-N-acetylgalactosaminyltransferase, GM2, GD2, GD3, MAGE-1, MAGE-2, MAGE-3, MUC-1, MUC-2, MUC-3, MUC-4, MUC-18, ICAM-1, C-CAM, V-CAM, ELAM, NM23, EGFR, E-cadherin, N-CAM, CEA, DCC, PSA, Iler2-neu, UTAA, melanoma antigen p75, K19, HKer 8, pMEL 17, tyrosine related proteins 1 and 2, p97, p53, RB, APC, DCC, NF-1, NF-2, WT-1, MEN-I, MEN-II, BRCA1, VHL, FCC, MCC, ras, myc, neu, raf, erb, src, fms, jun, trk, ret, gsp, hst, bcl, abil, C1q, C1r, C1s, C4,C2, Factor D, Factor B, properdin, C3, C5, C6, C7, C8, C9, C1Inh, Factor H, C4b-binding protein, DAF, membrane cofactor protein, anaphylatoxin inactivator S protein, HRF,MIRL, CR1, CR2, CR3, CR4, C3a/C4a receptor, HIV (gag, pol, gp41, gp120, vif, tat, rev, nef, vpr, vpu, vpx), HSV (ribonucleotide reductase, -TIF, ICP4, ICP8, ICP35, LAT-related proteins, gB, gC, gD, gE, gl, gJ), influenza (hemagglutinin, neuroaminidase, PB1, PB2, PA, NP, M<sub>1</sub>, M<sub>2</sub>, NS<sub>1</sub>, NS<sub>2</sub>), papillomaviruses (E1, E2, E3, E4, E5a, E5b, E6, E7, E8, L1, L2), adenovirus (E1A, E1B, E2, E3, E4, E5, L1, L2, L3, L4, L5), Epstein-Barr Virus (EBNA), Hepatitis B virus, (gp27<sup>s</sup>, gp36<sup>s</sup>, gp42<sup>s</sup>, p22<sup>c</sup>, pol, x) and nuclear matrix proteins.

Claim 22 (New) The method of claim 19, wherein said protein is selected from a group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IFN- $\alpha$ , IFN- $\beta$ , IFN- $\gamma$ , CD2, CD3,CD4, CD5, CD8, CD11A, CD11b, CD11c, CD16, CD18, CD21, CD28, CD32, CD34, CD35, CD40, CD44, CD45, CD54 CD56, K2, K1, P $\beta$ , O $\alpha$ , M $\alpha$ , M $\beta$ 2, M $\beta$ 1, LMP1, TAP2,

Appl. No. 09/552,461

Amdt. dated January 24, 2004

Reply to Office Action of Feb. 24, 2003

LMP7, TAP1, O $\beta$ , IA $\beta$ , IA $\alpha$ , IE $\beta$ , IE $\beta$ 2, IE $\alpha$ , CYP21, C4B, CYP21P, C4A, BF, C2, HSP, G7a/b, TNF- $\alpha$ , TNF- $\beta$ , D,L Qa, T1a, COL11A2, DP $\beta$ 2, DP $\alpha$ 2, DP $\beta$ 1, DP 1, DN 2, DQ 3, DQ 1, DQ DR , DR , HSP-70, HLA-B, HLA-C, HLA-X, HLA-E, HLA-J, HLA-A, HLA-H, HLA-G, HLA-F, NGF, somatotropin, somatomedins, parathormone, FSH, LH, EGF, TSH, TSH-releasing factor, HGH, GRHR, PDGF, IGF-I, IGF-II, TGF- $\beta$ , GM-CSF, M-CSF, G-CSF1, erythropoietin, -IICG, 4-N-acetylgalactosaminyltransferase, GM2, GD2, GD3, MAGE-1, MAGE-2, MAGE-3, MUC-1, MUC-2, MUC-3, MUC-4, MUC-18, ICAM-1, C-CAM, V-CAM, ELAM, NM23, EGFR, E-cadherin, N-CAM, CEA, DCC, PSA, Her2-neu, UTAA, melanoma antigen p75, K19, HKer 8, pMEL 17, tyrosine related proteins 1 and 2, p97, p53, RB, APC, DCC, NF-1, NF-2, WT-1, MEN-I, MEN-II, BRCA1, VHL, FCC, MCC, ras, myc, neu, raf, erb, src, fms, jun, trk, ret, gsp, hst, bcl, abil, C1q, C1r, C1s, C4, C2, Factor D, Factor B, properdin, C3, C5, C6, C7, C8, C9, C1Inh, Factor H, C4b-binding protein, DAF, membrane cofactor protein, anaphylatoxin inactivator S protein, HRF, MIF, CR1, CR2, CR3, CR4, C3a/C4a receptor, HIV (gag, pol, gp41, gp120, vif, tat, rev, nef, vpr, vpu, vpx), HSV (ribonucleotide reductase, -TIF, ICP4, ICP8, ICP35, LAT-related proteins, gB, gC, gD, gE, gI, gJ), influenza (hemagglutinin, neuroaminidase, PB1, PB2, PA, NP, M<sub>1</sub>, M<sub>2</sub>, NS<sub>1</sub>, NS<sub>2</sub>) papillomaviruses (E1, E2, E3, E4, E5a, E5b, E6, E7, E8, L1, L2), adenovirus (E1A, E1B, E2, E3, E4, E5, L1, L2, L3, L4, L5), Epstein-

Appl. No. 09/552,461

Amdt. dated January 24, 2004

Reply to Office Action of Feb. 24, 2003

Barr Virus (EBNA), Hepatitis B virus, (gp27<sup>s</sup>, gp36<sup>s</sup>, gp42<sup>s</sup>, p22<sup>c</sup>, pol, x)  
and nuclear matrix proteins.